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and the Effect of Their Inhibition on Survival of
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13. ABSTRACT (Maximum 200 Words) This research project investigates the role of the IkB kinase (IKK) and NF-κB in the development of prostate cancer (CaP), and examines the possibility that IKK inhibitors can be used in CaP treatment. To reach this goal we are employing two mouse models in which either the IKKβ or the IKKα subunit of IKK are deleted or inhibited in prostate epithelial cells. We found that neither IKKβ nor IKKα are required for normal prostate development, however IKKα may play an important role in the development of advanced CaP. The study on the role of IKKβ in prostate carcinogenesis in animal models is ongoing. We also found that IKK/NF-κB activities were increased during the evolution of androgen-independent CaP, a response that could be mediated by some of androgen-regulated genes, such as TMEFF2. Furthermore, we found that a prototypical IKK inhibitor IT-3 can suppress the proliferation of human CaP cells. Although a more thorough examination of the role of IKK/NF-κB in CaP development and progression is currently underway, our results obtained during last year suggest an important role for both IKKα and IKKβ in development and progression of CaP. Therefore inhibition of either protein kinase or both would be an effective and attractive option for the treatment of CaP.				
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The Role of I κ B kinase in prostate carcinogenesis and the effect of their inhibition on survival of prostate tumors

Introduction

NF- κ B transcription factors play an important role in regulation of innate and adaptive immune responses, inflammation, and cell survival (1). By virtue of their ability to activate many anti-apoptotic genes and become activated during chronic inflammation, NF- κ B transcription factors were suggested to play a key role in cancer development and progression, especially in the context of inflammation (2). A wide range of stimuli activate NF- κ B, mostly through I κ B kinase (IKK)-dependent phosphorylation and subsequent degradation of I κ B inhibitory proteins (3). The liberated NF- κ B dimers enter the nucleus where they enhance the transcription of many genes encoding cytokines, growth factors, cell adhesion molecules, and anti-apoptotic proteins (1). The IKK complex consists of two highly homologous kinase subunits (IKK α and IKK β) and a nonenzymatic regulatory component, IKK γ /NEMO (3).

There are two NF- κ B activation pathways. The first pathway, the classical NF- κ B activation pathway, is normally triggered in response to microbial and viral infections and exposure to proinflammatory cytokines which activate the three subunit IKK complex leading to phosphorylation-induced degradation of I κ Bs. This pathway depends mainly on the IKK β catalytic subunit (4). The other pathway, the alternative pathway, leads to activation of p52:RelB dimers by inducing processing of the NF- κ B2/p100 precursor protein that binds RelB in the cytoplasm. This pathway is triggered by certain members of the tumor necrosis factor (TNF) cytokine family through selective activation of IKK α homodimers by the upstream kinase NIK (3,5,6).

There is considerable evidence that the two IKK/NF- κ B signaling pathways are involved in carcinogenesis, cancer progression, metastasis and drug resistance. Although certain oncoviral proteins, cancer-associated chromosomal translocations, and mutations can lead to constitutive activation of NF- κ B in cancer progenitor cells (7), the most common mechanism leading to NF- κ B activation during tumorigenesis depends on autocrine and paracrine production of proinflammatory cytokines or chronic infections (2). Persistent activation of NF- κ B can lead to increased production of tumor growth factors by components of the tumor stroma as well as to upregulation of anti-apoptotic genes within the cancer cell itself (2). This process was recently demonstrated to occur during two different mouse models of inflammation-associated cancer leading to development of colorectal cancer (8) and hepatocellular carcinoma (9). We also provided evidence for a role of NF- κ B in inflammation-driven metastatic growth (10). In that model, as well, IKK-driven NF- κ B activation is responsible for production of growth and survival factors by stromal components (macrophages) and upregulation of anti-apoptotic genes within the cancer cell (10).

Prostate cancer (CaP) is one of the most common cancers in men and the second leading cause of cancer-related deaths among men in the United States. Androgens stimulate the growth of both the normal prostate epithelium and early CaP. While early CaP responds to androgen-ablation therapy, disease progression is characterized by the conversion from an androgen-dependent (AD) to androgen-independent (AI) phenotype (11). Although 80% of patients with advanced CaP initially respond to anti-androgen therapy, within 12-18 months most CaPs become AI, resulting in poor prognosis (12). The complex mechanism underlying the evolution

of AI cancer remains poorly understood, but its elucidation should offer ample opportunities for development of novel therapeutic measures.

It was shown that NF- κ B transcription factors can directly interact with several members of the nuclear receptor family including androgen receptor (AR) itself (13-15). Thus, NF- κ B may function as a co-activator for AR causing it to be active independently of androgen binding (13-15). In this case, prostate epithelial cells with high NF- κ B activity are rendered resistant to androgen withdrawal. However, NF- κ B can also trans-repress ligand-bound AR or repress expression of the AR gene itself (14). Most importantly, NF- κ B activity itself is repressed by androgen treatment via AR-mediated trans-repression (13,16) or other mechanisms. In this case, NF- κ B activity may increase in response to androgen withdrawal. Once activated, NF- κ B can stimulate production of various cytokines by prostate epithelial cells, CaP and stromal components. Specifically, IL-6, a well-known autocrine and paracrine growth factor for AI CaP cells (17,18), is encoded by a typical NF- κ B target gene (19). The dependence of IL-6 expression on NF- κ B was demonstrated in the prostate epithelium (16, 20). Importantly, the human AD CaP cell line, LNCaP, was shown to assume a neuroendocrine (NE) phenotype in response to IL-6 exposure (21). Although NE cancers of the prostate are rare, foci with NE-like features can be observed in nearly all prostate adenocarcinomas and extensive NE differentiation is generally considered to be of poor prognostic value (22). Indeed, NE differentiation appears more frequently in hormone-refractory cancer (22). Inhibition of IL-6 activity induces the regression of AI human CaP xenografts in mice (17). Thus, NF- κ B inhibition should result in a similar effect by inhibiting IL-6 expression. Studies performed on various CaP cell lines reveal that AI cells often display constitutive NF- κ B activity (23). Interestingly, inhibition of NF- κ B activity in such cells was achieved by treatment with ibuprofen, a non-steroidal anti-inflammatory drug (NSAID) that most likely acts as a non-specific IKK inhibitor in a manner akin to aspirin (23). Furthermore, constitutively activated NF- κ B in three different human CaP cell lines was linked to over expression of IKK subunits, and inhibition of NF- κ B activity in these cells through expression of a non-degradable super-repressor mutant of I κ B α resulted in either spontaneous apoptosis or increased sensitivity to TNF α (24).

In this ongoing research project we are focusing on the role of the I κ B kinase (IKK) complex and NF- κ B in the development of CaP, and are examining the possibility that IKK inhibitors can be used in the therapy of CaP through their ability to induce apoptosis of prostatic carcinoma cells or increase their susceptibility to apoptosis-inducing anti-cancer drugs or therapeutic radiation. After one year of extensive research we obtained very interesting and encouraging results that are summarized below.

Body:

- 1. Construct mice with a specific deletion of *Ikk β* in prostate epithelial cells and examine whether this deletion inhibits prostate carcinogenesis in the TRAMP model**
 - (1) Examine the role of IKK β in prostate carcinogenesis**

To test the role of the IKK β subunit in CaP development, we constructed a mouse strain that contain a prostate epithelium-specific deletion of the gene coding for the IKK β catalytic subunit. In these experiments we took advantage of *Ikk β ^{F/F}* mice, which carry a conditional loss-of-function “floxed” *Ikk β* allele. To delete IKK β in prostate epithelial cells we crossed *Ikk β ^{F/F}* mice to *PB-CRE4* transgenic mice, which express the CRE recombinase in prostate epithelial

cells (25). This yield an $Ikk\beta^{F/+}$ *PB-CRE4* heterozygote mice, which was intercrossed to generate homozygote $Ikk\beta^{F/F}$ *PB-CRE4* mice. We examined the efficiency of IKK β deletion in the prostate and in purified prostate epithelial cells from 10-12 week old male $Ikk\beta^{F/F}$ *PB-CRE4* mice by polymerase chain reaction (PCR) and immunoblotting and found efficient deletion of the $Ikk\beta^F$ allele and absence of IKK β protein in purified ventral and dorsolateral prostate gland epithelial cells from $Ikk\beta^{F/F}$ *PB-CRE4* mice (Fig 1). No differences in the size of the prostate gland and its histological composition between $Ikk\beta^{F/F}$ and $Ikk\beta^{F/F}$ *PB-CRE4* mice were observed. Thus, IKK β is not required for normal prostate development and maintenance. Since effective and prostate-specific deletion of IKK β has been confirmed, we crossed recombinant $Ikk\beta^{F/F}$ *PB-CRE4* mice as well as $Ikk\beta^{F/F}$ mice with the *TRAMP* transgenic mouse to generate $Ikk\beta^{F/F}$ *PB-CRE4-TRAMP* and $Ikk\beta^{F/F}$ *-TRAMP* mice. Cohorts of 15 male mice of the appropriate genotypes ($Ikk\beta^{F/F}$ *PB-CRE4-TRAMP* and $Ikk\beta^{F/F}$ *-TRAMP*) are being monitored for external signs of prostate cancer formation. Mice that exhibit large palpable tumors will be sacrificed and both primary and metastatic tumor tissues will be collected for primary cell culture, histological and biochemical analyses.

To examine the effect of IKK β deletion on prostate cancer progression, cohorts of age-matched 5-8 male mice of each genotype ($Ikk\beta^{F/F}$ *PB-CRE4-TRAMP* and $Ikk\beta^{F/F}$ *TRAMP*) are being prepared and will be sacrificed at 2, 3, 4, 5, 6 months of age. Size and weight of the prostate or CaP will be measured and recorded, and tissues will be subjected to detailed histological and biochemical analyses.

(2) Examine the role of IKK β in the evolution of androgen-independent prostate cancer

To examine whether IKK/NF- κ B is activated during the evolution of AI CaP, the androgen-dependent (AD) human CaP cell line LNCaP was inoculated subcutaneously into immunocompromised SCID mice. When tumor mass reached 1 cm³, one half of the tumor-bearing mouse cohort was sacrificed and AD tumor tissue was collected. The other half of the mouse cohort was castrated. Two months later when the tumors in the castrated mice re-grew, mice were sacrificed and tumor tissues were collected to yield AI tumors. Protein lysates derived from AD and AI tumors were assayed for NF- κ B DNA binding activity by an electrophoretic mobility shift assay (EMSA) (Fig 2a) and for IKK activity by an immunocomplex kinase assay (Fig 2b). The results clearly demonstrate that both NF- κ B and IKK activities are markedly elevated in AI tumor tissues (Fig 2).

It was reported that TMEFF2, an androgen-dependent gene, which is specifically expressed in brain and prostate, and exhibits antiproliferative effects on CaP (26). To test whether TMEFF2 may provide a link between development of AI and IKK-NF- κ B pathway, RNA was extracted from the tumors described above and expression of TMEFF2 mRNA was analyzed by real time PCR. TMEFF2 mRNA was decreased in AI tumors (Fig 3a). To test the effect of MEFF2 on NF- κ B transcriptional activity, the human CaP cell line DU-145, which has a high basal level of NF- κ B activity, was transfected with either a NF- κ B-dependent luciferance reporter plus different amount of TMEFF2 expression vector or the same reporter plus an empty expression vector as control. Extopic TMEFF2 expression inhibited NF- κ B activity in these cells (Fig 3b).

These results support the notion that the IKK-NF- κ B pathway may play an important role in development of AI CaP. To examine whether IKK β deletion in AD CaP cells can prevent or delay the evolution of AI CaP, the following experiments have been carried out or are ongoing. Primary mouse CaP cells were cultured from primary tumors isolated from 12-week-old

Ikk β ^{F/F}/TRAMP mice. These mice were generated by crossing TRAMP mice, which express SV40 T antigen in the prostate epithelium and develop CaP due to dysfunction of RB and p53 tumor suppressors with *Ikk β ^{F/F}* mice, which contain two “floxed” *Ikk β* alleles that can be deleted upon expression of Cre-recombinase (8). Colonies of CaP cells were formed two weeks after plating of the primary tumors, exhibiting low motility, high cell density and globular shape (Fig 4a). These colonies consisted mainly of small cells, which are thought to be cancer stem cells (27). These colonies were trypsinized and passed in culture to yield rapidly growing cells that formed invasive tumors when inoculated subcutaneously into immune compromised *Rag1*^{-/-} mice (Fig 4b). These cells expressed high levels of SV40-T antigen, p53 protein (presumably inactivated by T Ag) and normal levels of IKK α , IKK β and I κ B α protein (Fig 4c). After several rounds of IL-6 treatment, these cells have assumed a NE phenotype, which is characterized by rounding of cell bodies, development of long-branched neuritic-like processes, and presence of secretory vesicles (Fig 5a). These transdifferentiated cells express higher levels of the NE markers: Chromogranin-A (Fig 5b and 5c) and Synaptophysin (Fig 5b and 5d) and are more motile and dispersed than the original cells. The role of IKK β in this process, which can be evaluated upon deletion of the *Ikk β ^F* allele through Cre expression, is being examined.

To determine the consequence of IKK β inhibition on already developed tumors, especially the role of IKK β in the evolution of AI CaP, we are employing a transplant mouse model by manipulating the *Ikk β ^{F/F}/TRAMP* CaP cell lines that were established. *Ikk β ^{F/F}/TRAMP* cells will be infected with an adenovirus vector expressing Cre or with control “empty” viral vectors. Deletion of IKK β in transduced cells will be confirmed by immunoblotting and kinase assays. Equal number of IKK β -expressing and -nonexpressing CaP cells will be implanted subcutaneously into the right and flanks of *Rag1*^{-/-} mice, respectively (cohort of 7 mice). When the tumor will reach the size of 0.5 cm³, the host mice will be castrated, and tumor growth will be monitored. The times when tumors will re-emerge in the castrated mice and reach 1 cm³ in size will be recorded. Tumor tissues from primary and the metastatic sites will be collected for primary cell culture, histological and biochemical analyses.

It was reported that 80% TRAMP mice castrated at 12 weeks of age will develop aggressive AI CaP at 24 weeks of age (28). To determine the effect of IKK β ablation on development of androgen-independent prostate cancer (AI CaP) in TRAMP mice, cohorts of 20 male mice of each genotype (*Ikk β ^{F/F}/PB-CRE4-TRAMP* and *Ikk β ^{F/F}/TRAMP*) are being prepared and will be castrated at 12 weeks of age and sacrificed at 24 weeks of age. Size, weight and histology of the prostate tissue and of primary and metastatic CaPs will be measured and recorded. Prostate tissues, primary and metastatic CaP will be collected for primary cell culture, histological and biochemical analyses.

2 Examine whether inactivation of IKK α inhibits prostate carcinogenesis in the TRAMP model.

To investigate the role of the IKK α subunit, which may offer a more attractive target for drug development as it is not required for innate immune responses, TRAMP mice were crossed with *Ikk α ^{AA/AA}* mice which express a form of IKK α that can not be activated because two serines in its activation loop, which are phosphorylated by the upstream kinase NIK, were replaced with alanines (29). The resulting *Ikk α ^{AA/AA}/TRAMP* mice were monitored for tumor development and found to exhibit fewer metastases (including metastasis to lymph nodes and other organs) and survive longer than *WT/TRAMP* mice (Fig 6a). CaP in *Ikk α ^{AA/AA}/TRAMP* mice exhibit reduced cell proliferation (Fig 6b). However, there are no differences in size and weight of the prostate

gland between 12-week-old *Ikkα^{AA/AA}/TRAMP* and *WT/TRAMP* mice, suggesting that IKKα kinase activity is required for CaP progression but not for normal prostate development and early tumorigenesis.

To analyze the effect of IKKα kinase inhibition on development of AI CaP, cohorts of 20 male mice of each genotype (*Ikkα^{AA/AA}/TRAMP* and *WT/TRAMP*) that were castrated at 12 weeks of age will be sacrificed and analyzed at 24 weeks of age. The size and weight of primary and metastatic CaP will be measured and recorded. Normal prostate tissue, primary and metastatic tumors will be collected for histological, biochemical analyses and primary cell culture.

The stroma and extracellular matrix are essential for functional and morphological differentiation of the prostatic epithelium (30). It is also postulated that prostate stroma may play an important role in CaP development (31). Analysis of the role of IKKα either in prostate epithelial cells or stromal components in CaP development will provide us with important information as to the cell-type specificity of IKKα function. Since this can not be learned from *Ikkα^{AA/AA}* mice in which IKKα is inactivated in all cells, we plan to separate epithelial and stromal cells from mutant and WT CaPs. Equal number of epithelial cancer cells and stromal cells of different genotypes will be mixed in different combinations (*WT* stroma+*Ikkα^{AA/AA}/TRAMP* CaP; *Ikkα^{AA/AA}/TRAMP* stroma + *WT/TRAMP* CaP; etc). To examine the tumor development, equal numbers of cell combinations will be implanted under the renal capsule of SCID mice (5 mice for each cell combination). Two months later, mice will be sacrificed, and the size and weight of each tumor will be measured and recorded. Tumor tissues will be collected and analyzed as described above. To examine tumor development after androgen withdrawal, the same procedure will be repeated and one month after the cell transplantation mice will be castrated, and sacrificed two months later. Tumor size and weight will be measured and recorded, and tumor tissues will be analyzed.

3. Use various strategies to inhibit IKK activity and test them for their ability to inhibit proliferation and induce apoptosis in prostate cancer cell lines of human origin.

The experiments described above provide genetic tests for the involvement of IKKβ or IKKα in the development of CaP in mice. At the same time we are investigating the possible use of IKK/NF-κB inhibitors in the treatment of human CaP. This question is addressed through development of various IKK inhibitors, targeting either IKKα or IKKβ, and testing them for effects on human CaP cell lines, in vitro and mouse xenografts.

A prototypical IKK inhibitor named IT-3 was synthesized according to description in the patent literature by a chemist at UCSD cancer center. We examined IT-3 for its effects on the proliferation of several human CaP cell lines: DU145, PPC-1, PC3 and ALVA31. Previous work has shown that these cell lines exhibit constitutively activated NF-κB. The different cell lines were incubated in 96-well plates and treated with or without the inhibitor for 24 hours. Cell proliferation rates were determined by the MTT assay. We found that the IKK inhibitor IT3 can inhibit the proliferation of all human prostate cell lines tested (Fig 7). To assess effects on NF-κB activation, the CaP cell lines were pretreated with different concentrations of IT-3 before being stimulated with TNFα. Cells were collected at 30 minutes after TNFα treatment and IκBα levels were analyzed by immunoblotting. We found that IT-3 inhibited TNFα-induced IκBα protein degradation in all cell lines (Fig 8), suggesting that IT-3 does inhibit IKK activity. Further assays for the specificity of IT-3 action are underway.

At the same time other putative IKK/NF-κB inhibitors (the macrolides erythromycin,

clarithromycin, azithromycin, 10-deoxymethynolide, etc) are being tested for their inhibition of IKK and NF- κ B activities and their effects on the proliferation of CaP cell lines.

Those inhibitors that will be found to be effective in inhibiting the proliferation of CaP cells or capable of increasing their sensitivity to apoptosis-inducing drugs will be tested for their ability to exert these activities in xenograft models of human CaP. In these experiments two cell lines, PC-3 and DU-245, will be injected into each flank of a nude mouse to generate subcutaneous tumors. A week later, the mice will be subjected to intraperitoneal injections of the most effective inhibitors in each class or a vehicle control. These injections will be repeated two times a week and once the tumors in vehicle-treated animals will reach 1 cm in size, the mice will be sacrificed and tumor size and histology will be determined. To investigate whether the inhibitors increase tumor susceptibility to apoptosis-inducing drugs or radiation, we plan to repeat the experiments as described above and combine with IKK inhibitors with treatment with anti-cancer drugs or ionizing radiation.

Key Research Accomplishments

1. Specific deletion of IKK β in prostate epithelial cells was achieved by crossing *Ikk β ^{F/F}* mice to *PB-CRE4* transgenic mice to get *Ikk β ^{F/F}PB-CRE4* mice, which will provide a very good model for understanding the role of IKK β in prostate carcinogenesis and progression (Fig 1).
2. We found that neither IKK α nor IKK β is required for normal prostate development and maintenance, thus IKK β -specific or IKK α -specific inhibitors are unlikely to be toxic to the normal prostate.
3. We found that both IKK and NF- κ B activities are activated during the transition of CaP from an AD state to an AI state (Fig 2). Thus, IKK inhibitors may interfere with the increased aggressiveness of AI CaP.
4. We found that TMEFF2, an AD gene, is down-regulated in AI CaP, and its forced expression in the human CaP cell line DU-145 inhibits NF- κ B activity (Fig 3). Thus, TMEFF2 may be one of the molecules that link the emergence of AI CaP to IKK and NF- κ B activation.
5. We have established mouse CaP cell lines harboring *Ikk β ^{F/F}* alleles in which IKK β can be deleted upon expression of exogenous Cre protein (Fig 4). These cell lines will provide a convenient system for analyzing the effect of IKK β inhibition on tumor progression and response to chemotherapeutic drugs and radiation.
6. We found that mouse *Ikk β ^{F/F}* CaP cells can be induced to become neuroendocrine cells after IL-6 treatment (Fig 5). This provides us with a system for testing the role of IKK β in CaP progression.
7. We found that inhibition of IKK α kinase activity can delay the development and slow the growth of CaP in mice (Fig 6). Inhibition of IKK α also slow down the appearance of metastases. Thus specific IKK α inhibition may be effective in treatment of CaP in humans.
8. We found that a prototypical IKK inhibitor, IT-3, can suppress the proliferation of human prostate cancer cells (Fig 7 and 8). This inhibitor may serve as a starting point for the development of IKK α - and IKK β -specific inhibitors.

Reportable Outcomes

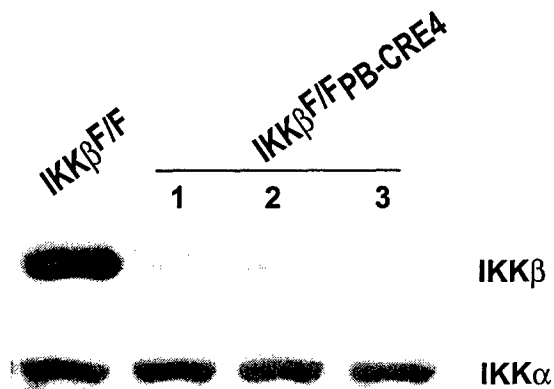


Fig 1, IKK β protein is efficiently deleted in prostate epithelial cells in IKK $\beta^{F/F}$ PB-CRE4 mice. Purified ventral and dorsolateral prostate epithelial cells from 10-12 week old male IKK $\beta^{F/F}$ and IKK $\beta^{F/F}$ PB-CRE4 mice were lysed for immunoblot analysis. The results show efficient deletion of IKK β in ventral and dorsolateral prostate epithelial cells from IKK $\beta^{F/F}$ PB-CRE4 mice .

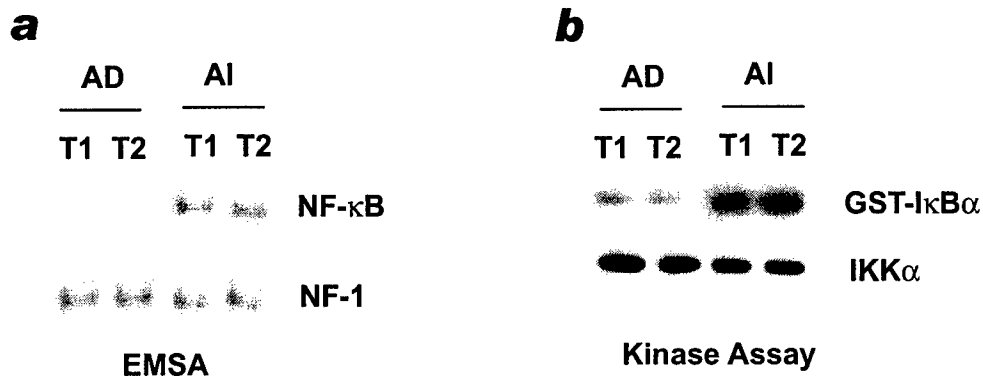


Fig 2, Human LNCaP cells were implanted subcutaneously into SCID mice. When the tumor mass reached 1 cm³, half of the tumor-bearing mice were sacrificed and tumor tissues were collected (androgen-dependent tumors, AD). The remaining half were castrated. Two months later when the tumors in the castrated mice regrew, the mice were sacrificed and tumors were collected (androgen-independent tumors, AI). Tumor extracts were used for electrophoretic mobility shift assay (EMSA) (**a**) and determining of IKK kinase activity (**b**). Both NF-κB DNA binding activity and IKK kinase activity were markedly increased in AI tumors as compared to AD tumors.

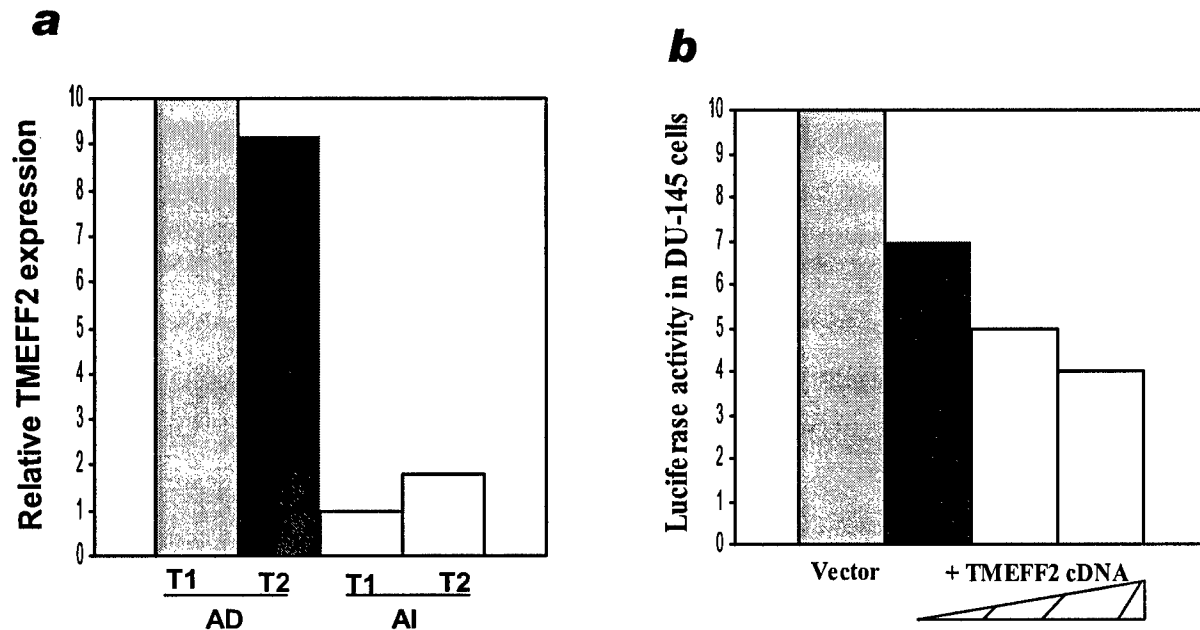


Fig 3, **(a)** RNA extracted from AD and AI tumors described in Figure 2 was used in Real Time RT-PR analysis to examine expression of TMEFF2. **(b)** Human DU-145 cells were transfected with either NF- κ B luciferance reporter + different amounts of TMEFF2 expression vector or reporter + empty expression vector as a control. **(b)** The results are relative luciferance activity.

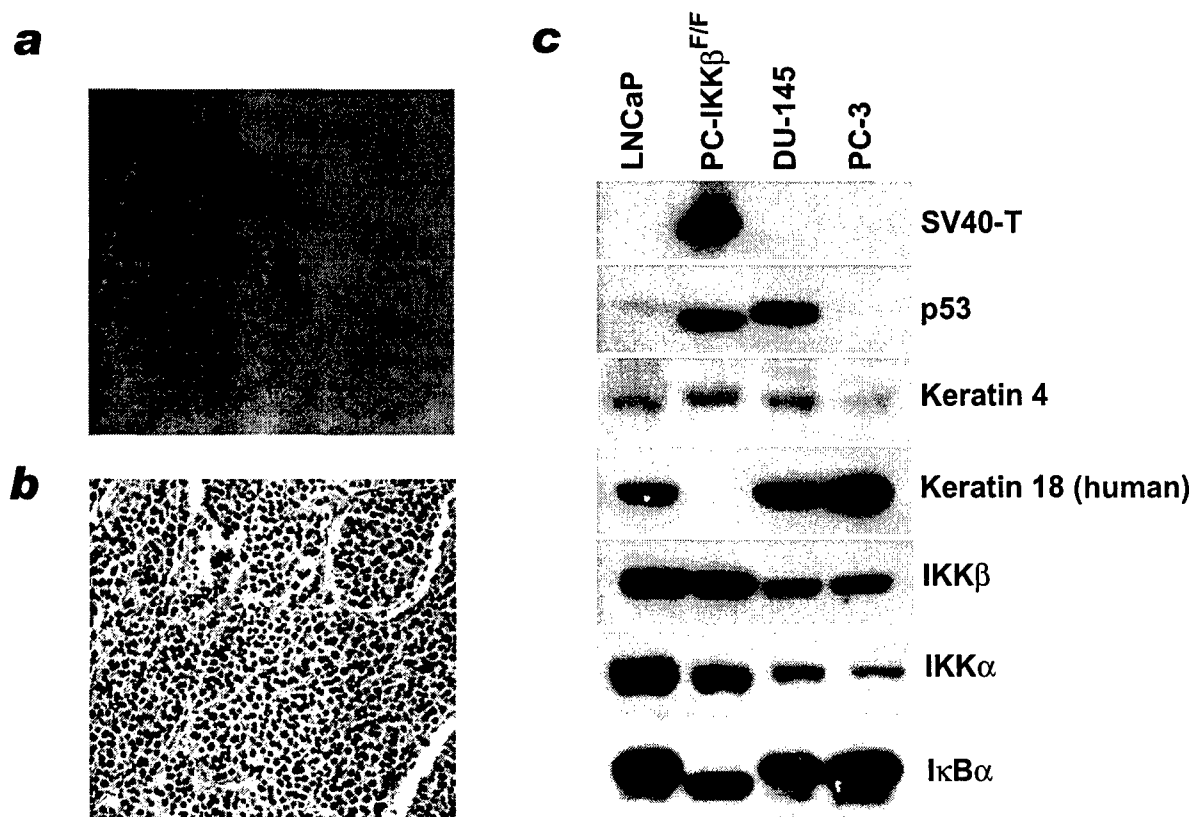


Fig 4, Primary cultures of mouse CaP cells were derived from a single primary prostate tumor isolated from IKK β ^{F/F}/TRAMP mice. Colonies were formed after two weeks. Large colonies with high cell density and globular shape, consisting mainly of small cells, were picked up for further passage (**a**). These cells grew aggressively and formed invasive tumors when subcutaneously inoculated into Rag1^{-/-} mice. (**b**) The histology of the tumors formed by these cells. Tumors were paraffin embedded, H&E stained and examined under microscope (x 20). (**c**) These cells expressed high levels of V40-T, p53 protein and normal levels of IKK β , IKK α and I κ B α protein, relative to human CaP cell lines. Protein expression was examined by immunoblot analysis.

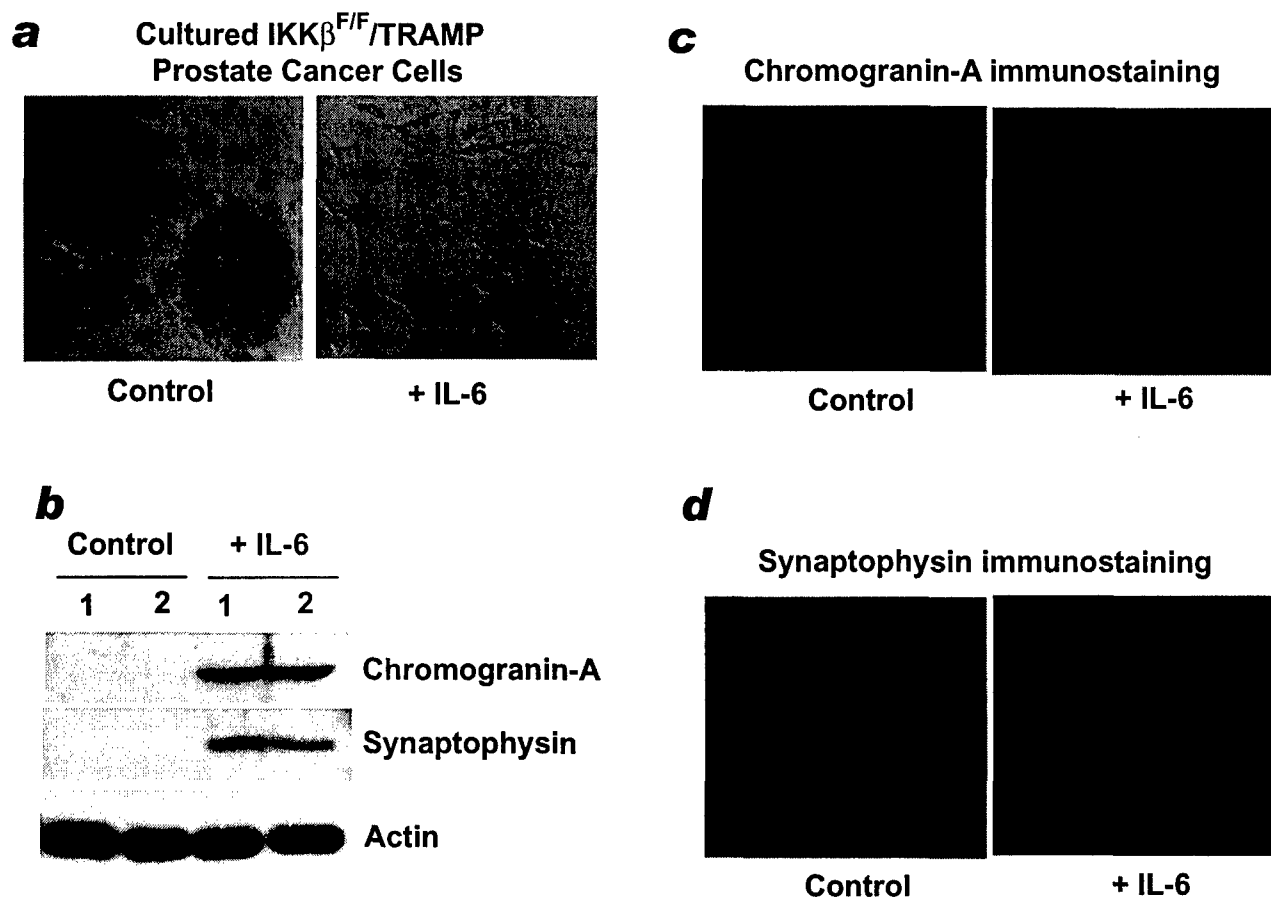


Fig 5, Cultured IKK $\beta^{F/F}$ /TRAMP CaP cells were incubated with or without mouse IL-6. After several passages, the IL-6 treated cells have assumed a neuroendocrine (NE) cell phenotype. These cells are characterized by rounding of cell bodies, development of long-branched neuritic-like processes, and the presence of secretory vesicles (**a**). These transdifferentiated cells express higher levels of the NE markers Chromogranin-A (**b, c**) and Synaptophysin (**b, d**) detected by either immunoblot analysis (**b**) or indirect immunofluorescence after staining with specific antibodies (**c, d**).

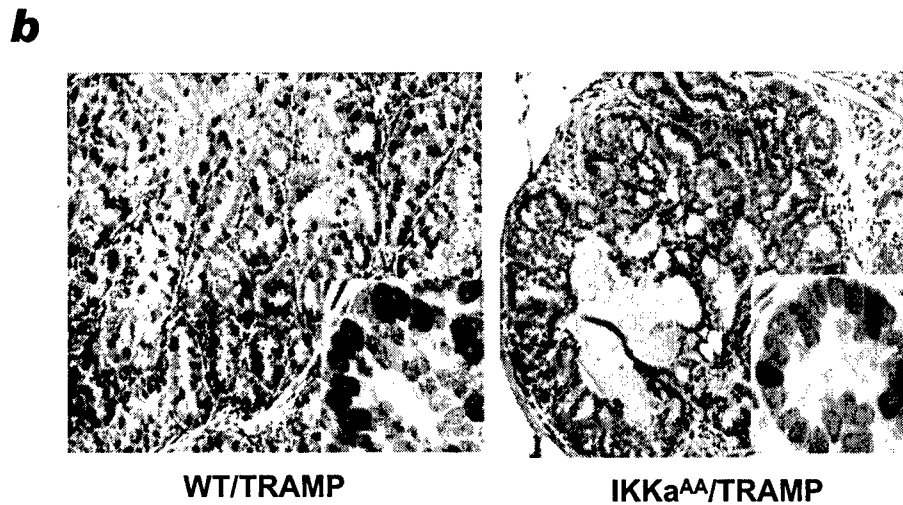
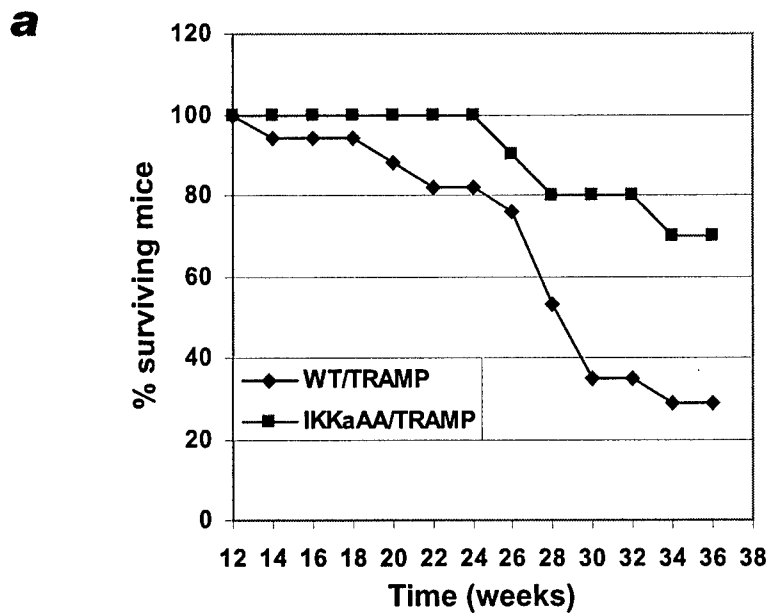


Fig 6, (**a**) Comparison of survival between WT/TRAMP (n=23) and IKK α^{AA} /TRAMP (n=19) mice. IKK α^{AA} /TRAMP mice survive much longer than WT/TRAMP mice. (**b**) Five months old mice were pulsed with 5-bromodeoxyuridine (BrdU) and sacrificed. BrdU incorporation into Caps and adjacent normal tissues, which identifies cells undergoing DNA synthesis, was examined by staining with anti-BrdU antibody.

MTT ASSAY

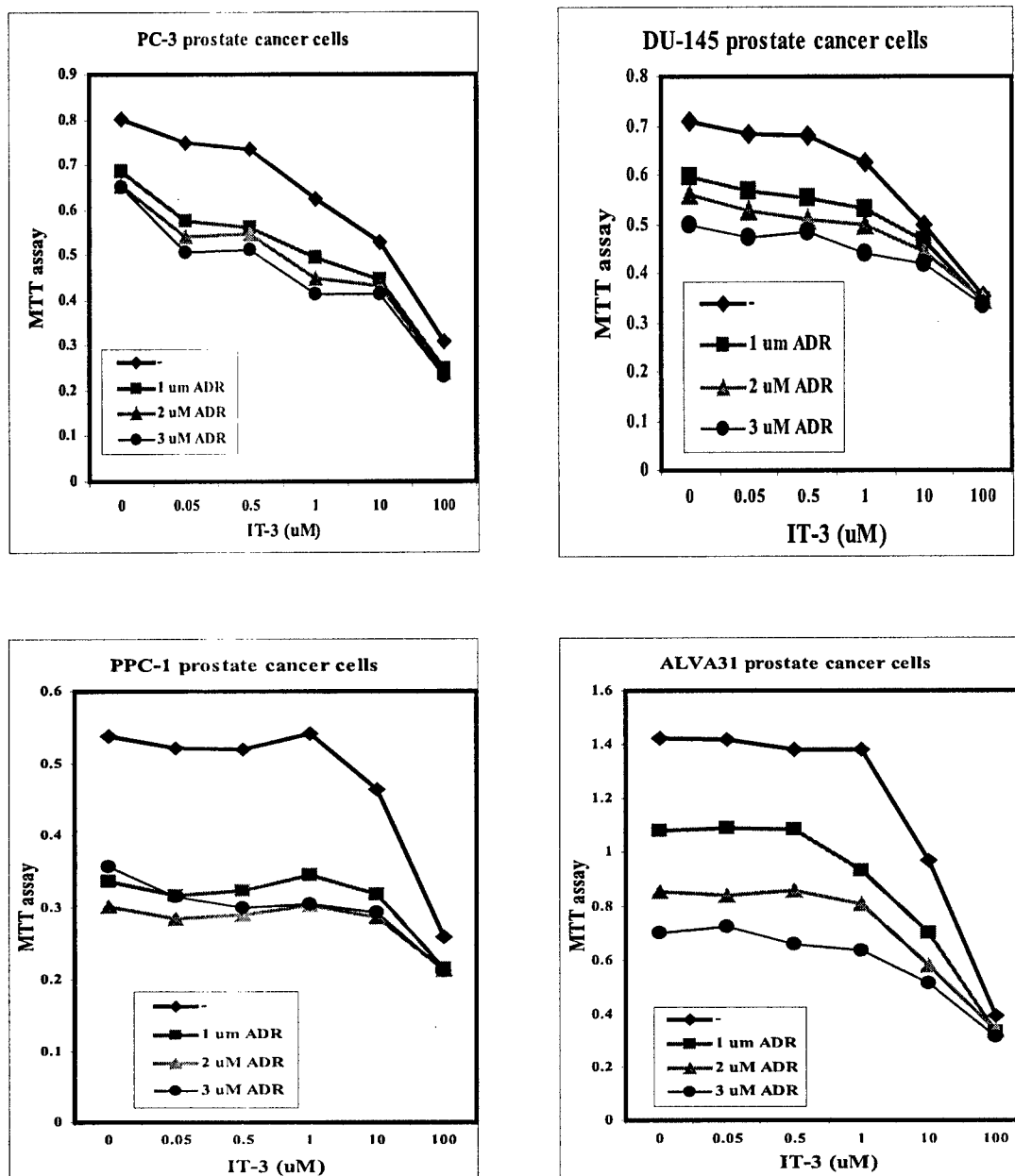


Fig 7 The IKK inhibitor IT-3 inhibits human CaP cell proliferation and or viability. Four human CaP cell lines (DU-145, ALVA31, PC-3 and PPC-1) as indicated were treated with IT-3, alone or in combination with adriamycin (ADR) for 24 hours. Cell viability was measured by the MTT assay. The results show that IT-3 can inhibit the proliferation or reduce viability of human CaP cells.

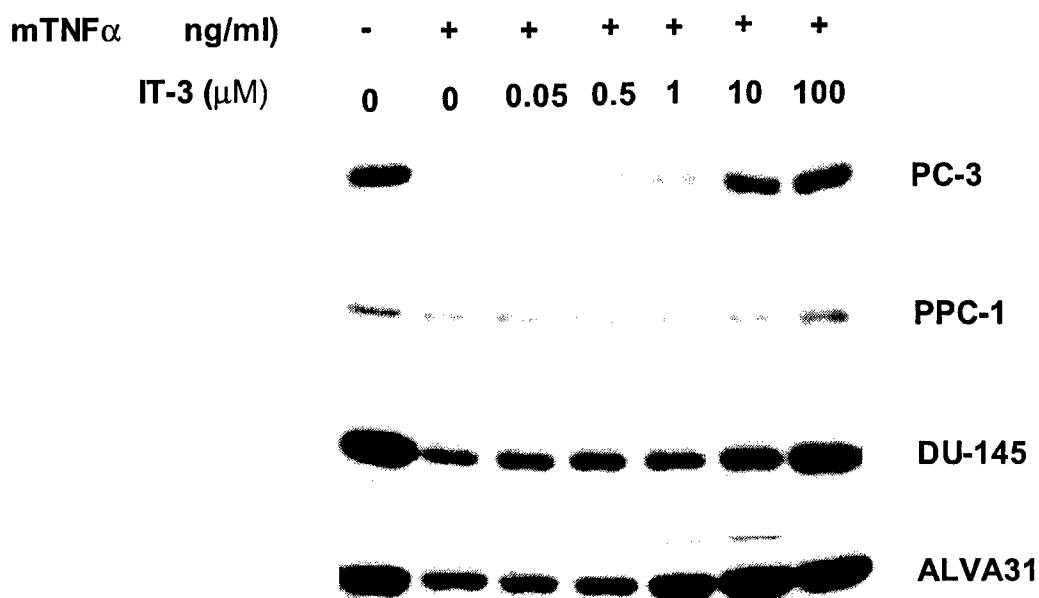


Fig 8 Immunoblot analysis of I κ B α levels in different CaPcell lines. Cells were pretreated with different concentrations of the IKK inhibitor IT-3 before TNF α stimulation. Cells were collected at 30 minutes after TNF α treatment, and I κ B α levels were examined by immunoblotting. IKK activation by TNF α induces the degradation of I κ B α .

Conclusions

In summary, our results obtained during the first year of the project suggest that both IKK α and IKK β may play an important role in development and progression of human and mouse CaP. Clearly, a more thorough examination of the role of the two IKK subunits in CaP development and progression is needed, especially during the transition of CaP from an AD to an AI state. These functions are examined in our ongoing research. We have also obtained encouraging results that a prototypical IKK inhibitor can inhibit the proliferation or induce the death of human CaP cell lines.

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